Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of ERK2 [2 – 360]

Enzyme description:- ERK2 [2 – 360]

Clone number:- DU 650

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 68, 721.20 daltons
Average Mass 68, 765.46daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.28

Purity:- >90 %

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 50 % glycerol, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -20 °C

Assay:- Standard filter binding assay

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 % 2–mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-
Myelin basic protein Final concentration: 0.3 mg/ml

Clone Data Sheet

ERK2 [2 - 360]

Protein	ERK2 [2 – 360]
Clone number	DU 650
Species	Human
Accession number	NM_002745
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEG DKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIA DKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKD FETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPD FMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQID KYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGP LGSPNSRVD**AAAAAAGAGPEMVRGQVFDVGPRYTNLSY** **IGEGAYGMVCSAYDNVNKVRVAIKKISPFEHQTYCQRT** **LREIKILLRFRHENIIGINDIIRAPTIEQMKDVYIVQD** **LMETDLYKLLKTQHLSNDHICYFLYQILRGLKYIHSAN** **VLHRDLKPSNLLLNTTCDLKICDFGLARVADPDHDHTG** **FLTEYVATRWYRAPEIMLNSKGYTKSIDIWSVGCILAE** **MLSNRPIFPGKHYLDQLKHILGILGSPSQEDLNCIINL** **KARNYLLSLPHKNKVPWNRLFPNADSKALDLLDKMLTF** **NPHKRIEVEQALAHPYLEQYYDPSDEPIAEAPFKFDME** **LDDLPKEKLKELIFEETARFQPGYRS**
Native sequence	Amino acids A2 – S360 (end) of human p42MAPK. Residue A238 of the fusion protein is equivalent to A2 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFQGPL) residues 221 - 229

Cloning sites *Sal*1 and *Not*1 of pGEX6P-3

Nucleotide sequence of insert

GCGTCGACGCGGCGGCGGCGGCGGGCGCGGGCCCGGAGATGG
TCCGCGGGCAGGTGTTCGACGTGGGGCCGCGCTACACCAACCTCT
CGTACATCGGCGAGGGCGCCTACGGCATGGTGTGCTCTGCTTATG
ATAATGTCAACAAAGTTCGAGTAGCTATCAAGAAAATCAGCCCCT
TTGAGCACCAGACCTACTGCCAGAGAACCCTGAGGGAGATAAAAA
TCTTACTGCGCTTCAGACATGAGAACATCATTGGAATCAATGACA
TTATTCGAGCACCAACCATCGAGCAAATGAAAGATGTATATATAG
TACAGGACCTCATGGAAACAGATCTTTACAAGCTCTTGAAGACAC
AACACCTCAGCAATGACCATATCTGCTATTTTCTCTACCAGATCC
TCAGAGGGTTAAAATATATCCATTCAGCTAACGTTCTGCACCGTG
ACCTCAAGCCTTCCAACCTGCTGCTCAACACCACCTGTGATCTCA
AGATCTGTGACTTTGGCCTGGCCCGTGTTGCAGATCCAGACCATG
ATCACACAGGGTTCCTGACAGAATATGTGGCCACACGTTGGTACA
GGGCTCCAGAAATTATGTTGAATTCCAAGGGCTACACCAAGTCCA
TTGATATTTGGTCTGTAGGCTGCATTCTGGCAGAAATGCTTTCTA
ACAGGCCCATCTTTCCAGGGAAGCATTATCTTGACCAGCTGAAAC
ACATTTTGGGTATTCTTGGATCCCCATCACAAGAAGACCTGAATT
GTATAATAAATTTAAAAGCTAGGAACTATTTGCTTTCTCTTCCAC
ACAAAAATAAGGTGCCATGGAACAGGCTGTTCCCAAATGCTGACT
CCAAAGCTCTGGACTTATTGGACAAAATGTTGACATTCAACCCAC
ACAAGAGGATTGAAGTAGAACAGGCTCTGGCCCACCCATATCTGG
AGCAGTATTACGACCCGAGTGACGAGCCCATCGCCGAAGCACCAT
TCAAGTTCGACATGGAATTGGATGACTTGCCTAAGGAAAAGCTCA
AAGAACTAATTTTTGAAGAGACTGCTAGATTCCAGCCAGGATACA
GATCTtaagcggccgcgc